Supplemental Material

High Content Phenotypic Profiling in Oesophageal Adenocarcinoma Identifies Selectively Active Pharmacological Classes of Drugs for Repurposing and Chemical Starting Points for Novel Drug Discovery

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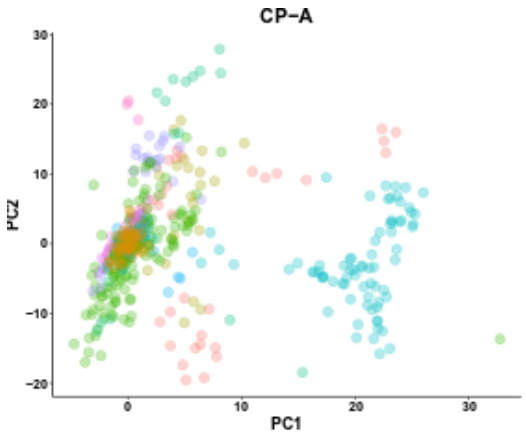
Supplementary Table S1. Compound Libraries and screening concentrations.

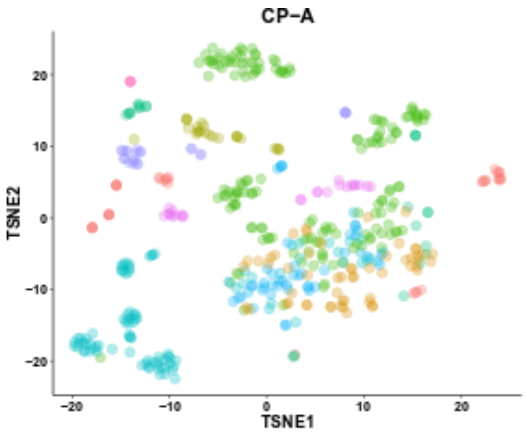
Library	**Concentration (μM)**
Prestwick Chemical Library	1
BioAscent 3K Library	10
LOPAC	3
Bespoke Library	1-3
CRUK therapeutics discovery laboratories Library	10-12

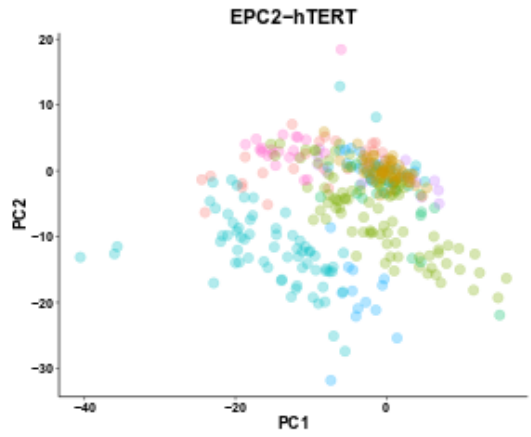
Supplementary Table S2. Reference Library of Compounds. 37 compounds and their classes.

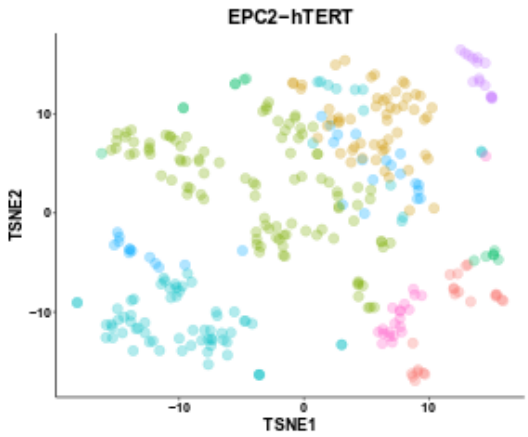
Compound	**Mechanism of Action**
Cytochalasin B	Actin disrupting
Cytochalasin D	Actin disrupting
Latrunculin	Actin disrupting
Camptothecin	DNA damaging
SN38	DNA damaging
Dasatinib	Kinase inhibitor

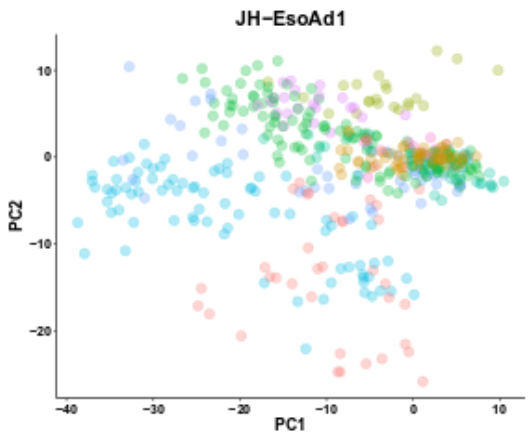
Saracatinib	Kinase inhibitor
Epothilone B	Microtubule disrupting
Paclitaxel	Microtubule disrupting
Colchicine	Microtubule disrupting
Nocodazole	Microtubule disrupting
Monastrol	Microtubule disrupting
ARQ621	Microtubule disrupting
Barasertib	Microtubule disrupting
ZM447439	Microtubule disrupting
MG132	Protein degradation
Lactacystin	Protein degradation
ALLN	Protein degradation
ALLM	Protein degradation
Cycloheximide	Protein synthesis
Emetine	Protein synthesis
Lovastatin	Statin
Simvastatin	Statin
SAHA	HDAC inhibitor
Panobinostat	HDAC inhibitor
Trichostatin A	HDAC inhibitor
Romidepsin	HDAC inhibitor
Entinostat	HDAC inhibitor
Quisinostat	HDAC inhibitor
Ricolinostat	HDAC inhibitor
Tubastatin A	HDAC inhibitor
Droxinostat	HDAC inhibitor
PCI34051	HDAC inhibitor
TMP195	HDAC inhibitor
LMK235	HDAC inhibitor
CUDC907	HDAC inhibitor
Belinostat	HDAC inhibitor

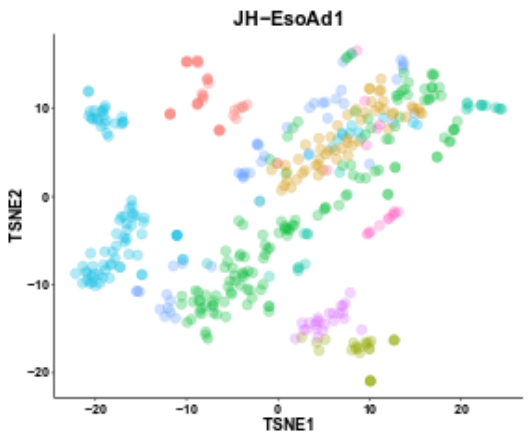

CP-A
PC2
PC1

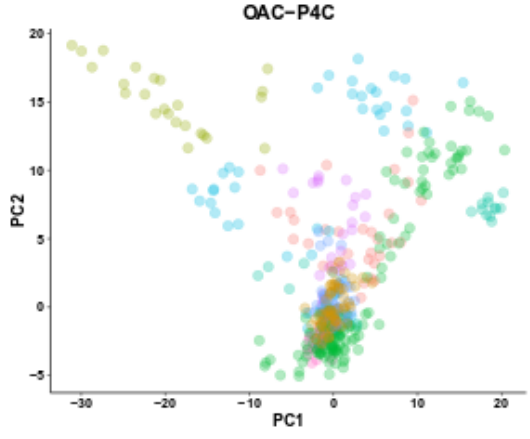

CP-A
TSNE2
TSNE1

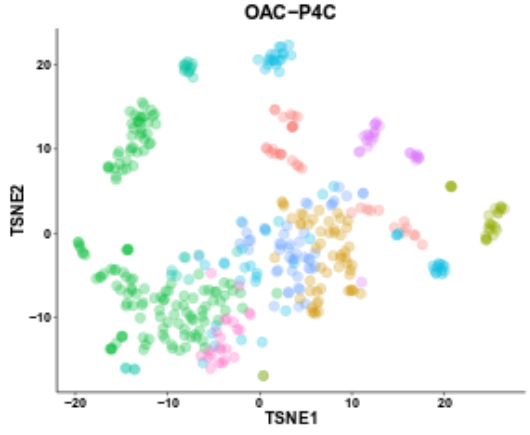

EPC2-hTERT
PC2
PC1

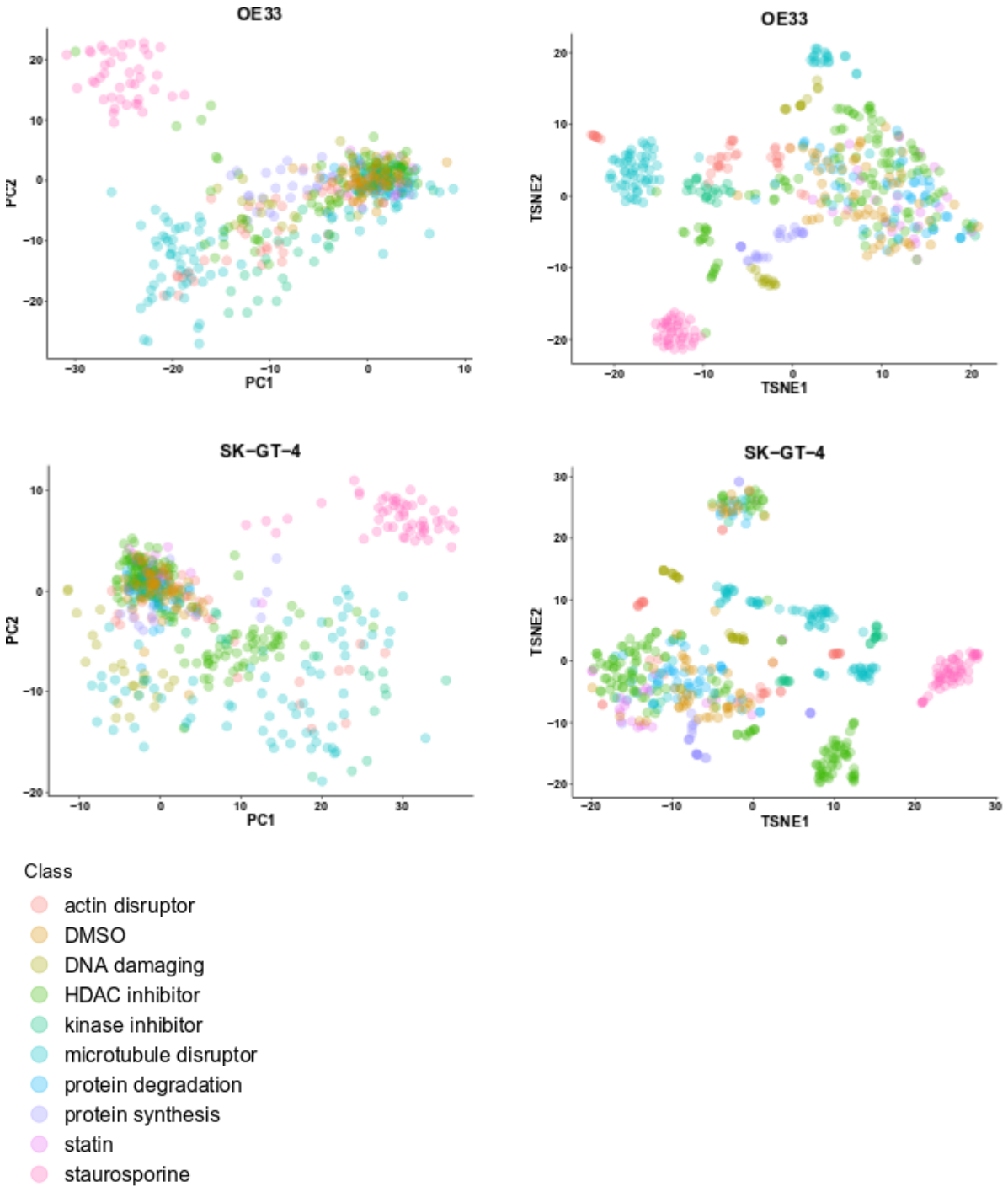

EPC2-hTERT
TSNE2
TSNE1


JH-EsoAd1
PC2
PC1

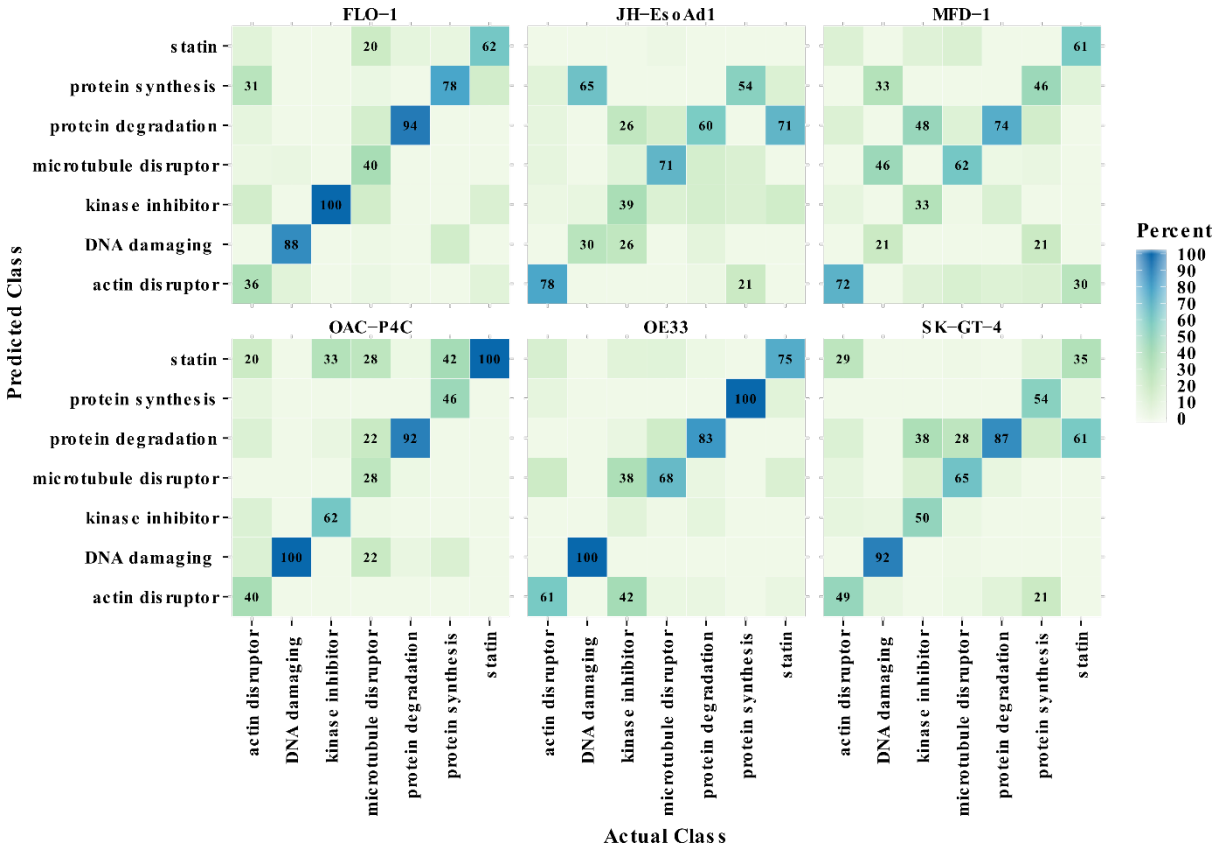

JH-EsoAd1
TSNE2
TSNE1


OAC-P4C
PC2
PC1


OAC-P4C
TSNE2
TSNE1

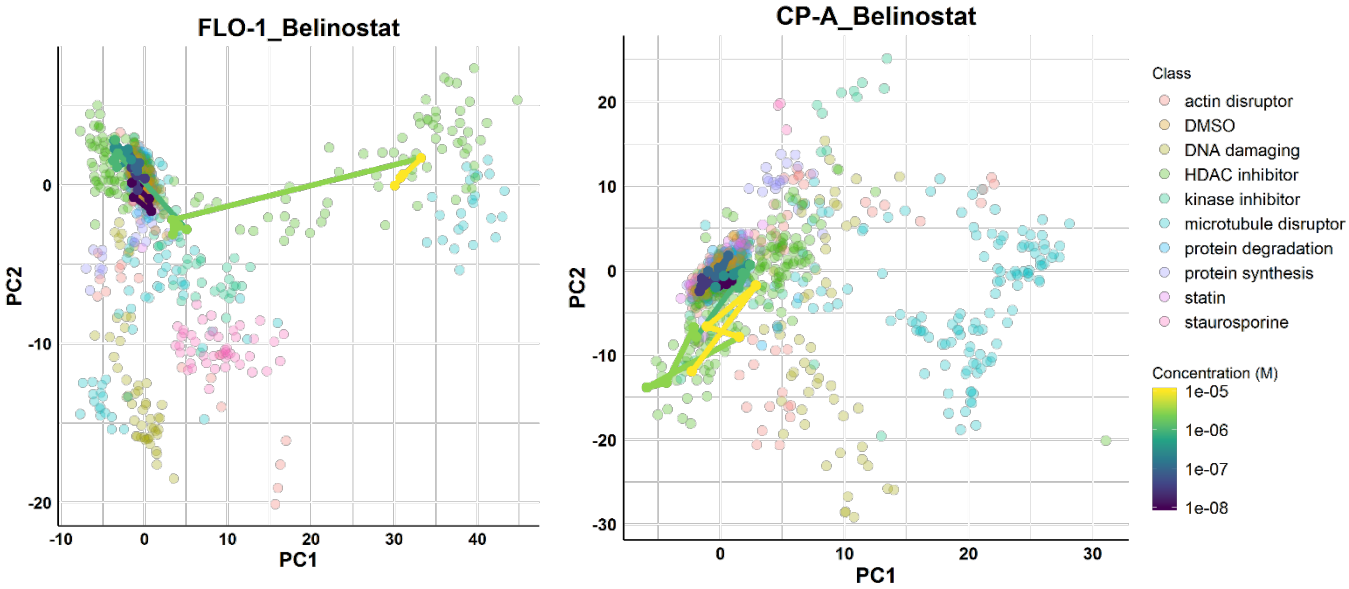


Supplementary Fig. S1. PCA and t-SNE plots. The first two components of principal component analysis (PCA) and t-distributed stochastic neighbour embedding (T-SNE) for the reference library compound treatments for the cell panel (excluding the FLO-1 and MDF-1 cell lines, see Figure 2). Points are coloured by mechanistic class and multiple compound concentrations are plotted.

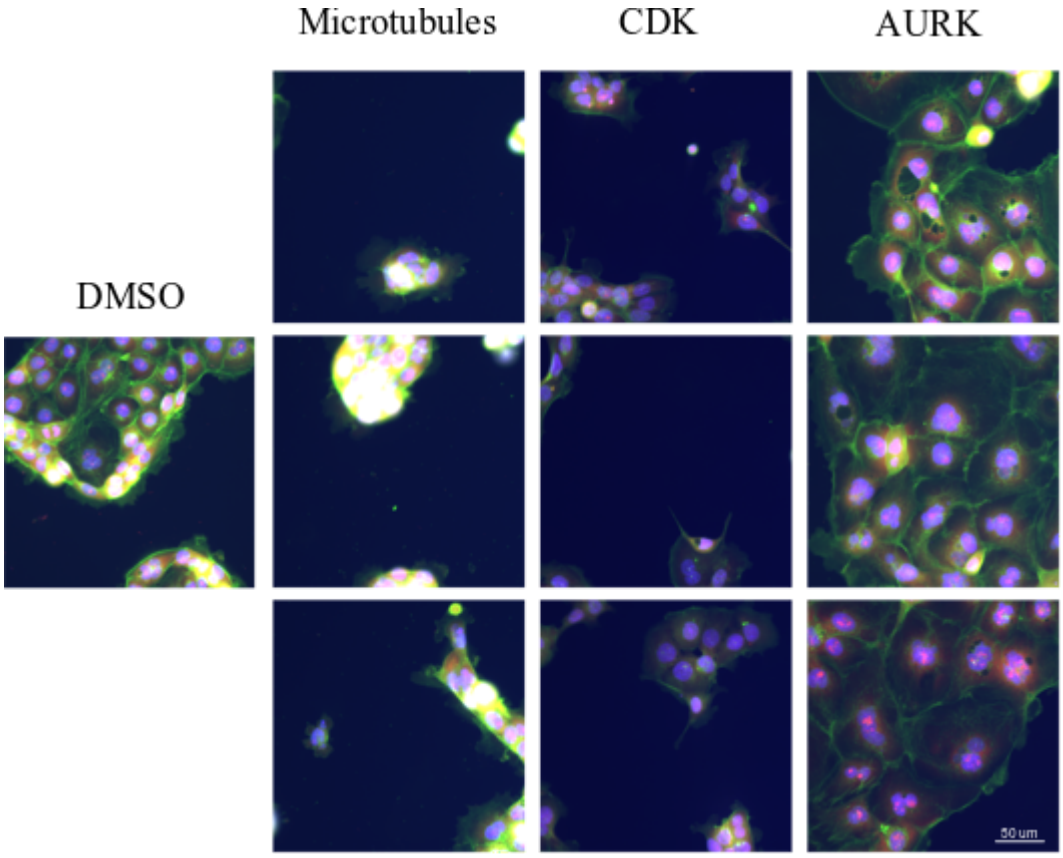


Supplementary Fig. S2. Leave-one-out random forest confusion matrices for reference library of compounds with known mechanisms-of-action. Prediction accuracies for each withheld cell line from a random forest classifier trained on the other five cell lines at a time.

A



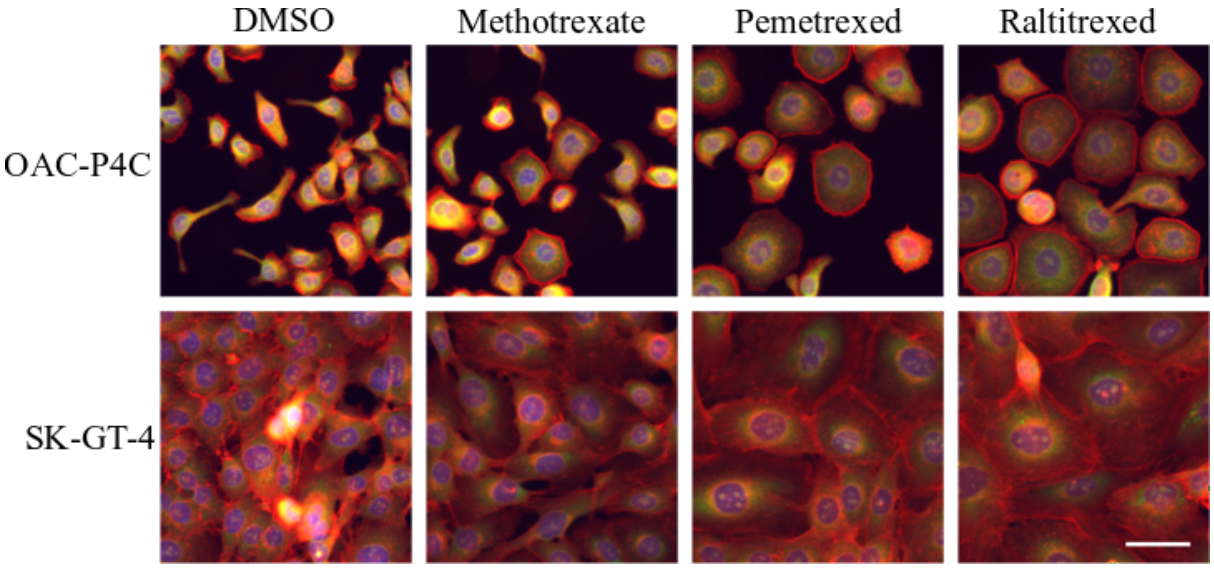
B



Supplementary Fig. S3. Phenotypic analysis Data. A) Phenotypic dose response for HDAC inhibitor Belinostat. The first two principal components for the feature data from the Belinostat dose response overlaid on reference library for FLO-1 and CP-A cell lines. B) Colour combined images for JH-EsoAD1 cells treated with three compounds from each of three classes; Aurora kinase (AURK) inhibitors, cyclin dependent kinase (CDK) inhibitors, Microtubule disruptors. DAPI channel (blue), TxRED channel (green), Cy3 channel (red). Scale bar is 50 μm.

Supplementary Table S3. Antimetabolite IC50s across the panel of cell lines (nM).

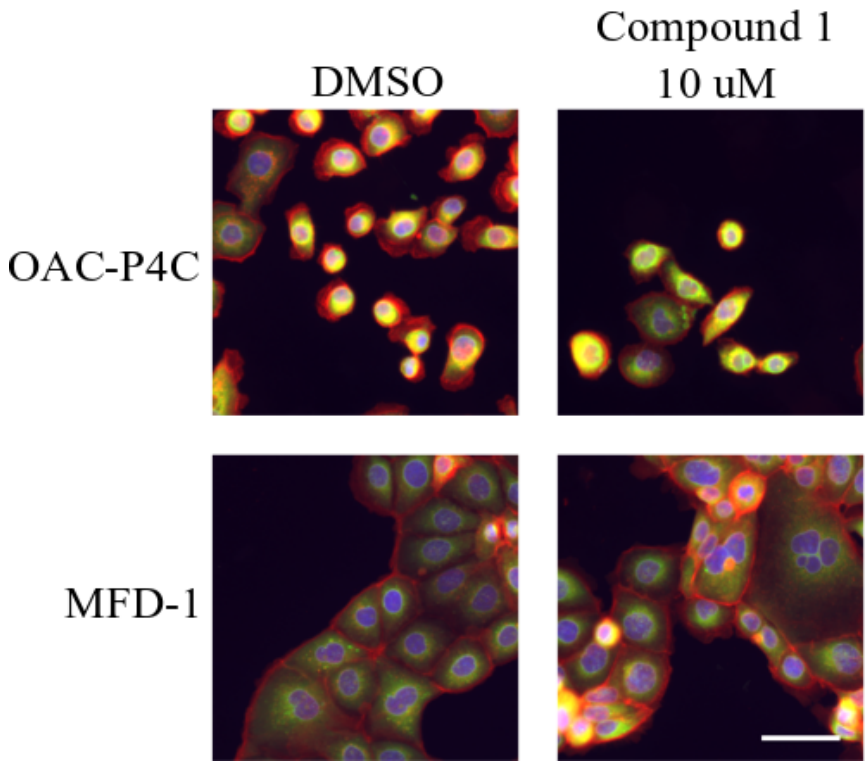
Cell Line	Methotrexate	Pemetrexed	Raltitrexed
JH-EsoAD1	99	110	9
FLO-1	74	224	12
MFD-1	112	263	30
OE33	52	87	4
OAC-P4C	421	10000	31
SK-GT-4	120	870	17



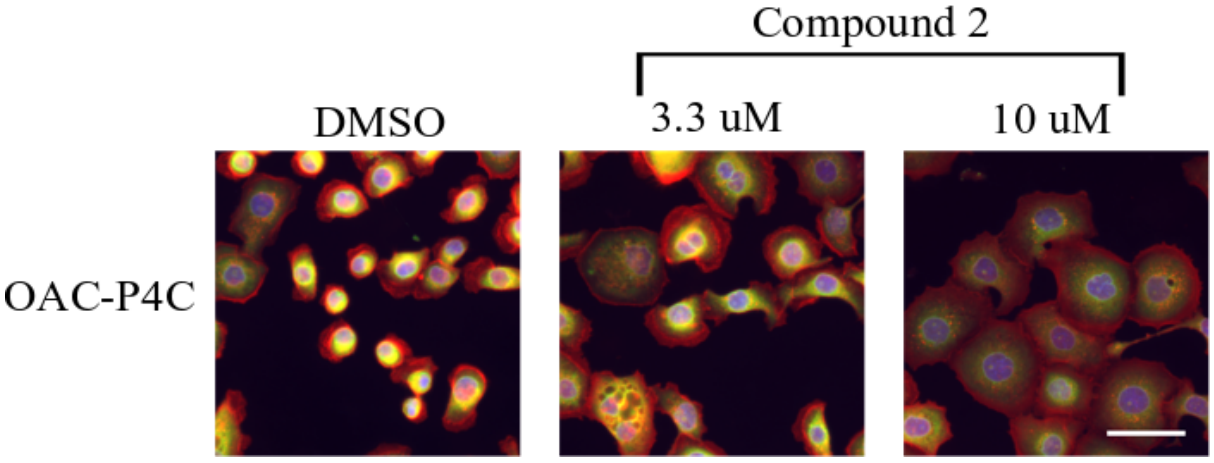
Supplementary Fig. S4. Colour combined images for the antimetabolites Methotrexate, Pemetrexed and Raltitrexed at 10μM in two exemplar cell lines, OAC-P4C and SK-GT-4. Scale bar 50 μm. DAPI channel (blue), TxRED channel (red), FITC channel (Green).

Supplementary Table S4. NanoString normalised counts for Histone H3 subunits. MTX = Methotrexate.

	CPA DMSO	CPA MTX	EPC2 DMSO	EPC2 MTX	FLO1 DMSO	FLO1 MTX	OE33 DMSO	OE33 MTX	SKGT4 DMSO	SKGT4 MTX
HIST1H3H	30,333	29,853	19,088	18,171	18,179	7,925	24,946	9,563	25,135	13,495
HIST1H3G	17,566	17,337	10,793	10,095	17,970	9,243	14,998	5,527	17,573	10,035
HIST1H3B	26,664	25,856	17,285	16,441	28,459	18,330	30,594	15,909	27,481	17,485



Supplementary Fig. S5. Colour combined images for Compound 1 at 10μM in the two most sensitive cell lines, OAC-P4C and MFD-1. DMSO images included for comparison. Scale bar 50 μm. DAPI channel (blue), TxRED channel (red), FITC channel (Green).



Supplementary Fig. S6. Supplementary Fig. S5. Colour combined images for Compound 2 at 3.3 and 10μM in the most sensitive cell line, OAC-P4C. DMSO image included for comparison. Scale bar 50 μm. DAPI channel (blue), TxRED channel (red), FITC channel (Green).